PB81-240848

The Occurrence of Microorganisms in Water Main Encrustations

(U.S.) Municipal Environmental Research Lab.
Cincinnati, OH

Jul 81

U.S. Department of Commerce
National Technical Information Service
NTIS.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. REC
EPA-600/J-80-322	Journal Article	PB81-240848

4. TITLE AND SUBTITLE
The Occurrence of Microorganisms in Water Main Encrustations

5. REPORT DATE
July 1981

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Martin J. Allen, Raymond H. Taylor, and Edwin E. Geldreich

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Municipal Environmental Research Laboratory--Cin, OH
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

10. PROGRAM ELEMENT NO.
BNC1A

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Municipal Environmental Research Laboratory--Cin, OH
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA/600/MERL

15. SUPPLEMENTARY NOTES

16. ABSTRACT
Colonization of microorganisms and resultant high bacterial populations often occur on or within protective encrustations or tubercules inside a distribution system. Formation of these encrustations is affected by a variety of physical, chemical, and biological activities within the pipes, which can be examined in situ by scanning electron microscopy (SEM). SEM photographs demonstrate graphically the survival of microorganisms in the distribution system and their possible role in tubercle formation.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

PRODUCT OF:
NATIONAL TECHNICAL INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
	20. SECURITY CLASS *(This page)*	22. PRICE

EPA Form 2220-1 (9-73)

RESEARCH AND TECHNOLOGY

EPA-600/J-80-322
Journal Article

The occurrence of microorganisms in water main encrustations

Martin J. Allen, Raymond H. Taylor, and Edwin E. Geldreich

Colonization of microorganisms and resultant high bacterial populations often occur on or within protective encrustations or tubercules inside a distribution system. Formation of these encrustations is affected by a variety of physical, chemical, and biological activities within the pipes, which can be examined in situ by scanning electron microscopy (SEM). SEM photographs demonstrate graphically the survival of microorganisms in the distribution system and their possible role in tubercle formation.

Potable waters being transported through distribution systems are subject to both chemical and microbial quality changes. Specifically related to microbial activities during distribution are the development of taste and odors, increased turbidity, and the regrowth of bacteria surviving the treatment processes. Some organisms associated with finished water deterioration include *Pseudomonas, Flavobacterium, Achromobacter, Proteus, Klebsiella, Bacillus, Serratia, Corynebacterium, Gallionella,* and *Arthrobacter.*[1] A significant regrowth of these and other organisms can result in the desensitization of coliform detection methodologies[2] and possibly an increased health risk.[3] Thus the factors affecting microbial quality changes in potable water during distribution have been the subject of numerous studies.[4-9] Such investigations have provided insight as to which variables significantly affect finished water quality.

The parameters most often cited as being associated with bacterial changes are the chemical-biological quality of the treated water, the effectiveness and concentration of residual disinfectants, the physical integrity of the distribution network, water temperature, and residence time of the water. While improvement of such water treatment processes as organics removal or efficacious application of disinfectants does minimize microbial activities, the condition of the distribution system continues to strongly influence changes in the microbiological quality of the finished water. These system-mediated quality changes, which affect corrosion and sediment deposition, appear to be related to the ability of microorganisms to colonize within encrustations on the interior surfaces of the distribution pipes. The formation of these encrustations is, of course, further affected by flow rates, chlorine residuals, nutrient availability, and pH. This study sought to determine whether such encrustations contribute to bacterial regrowth in finished water systems.

Previously reported studies[10-13] have noted that water main deposits—encrustations and tubercles—contain appreciably higher bacterial populations than are present in the distribution waters. These studies found that when encrustations obtained from a variety of systems were sectionally analyzed for microorganisms, the highest populations were always located at the outer surface of the specimen, which was in continuous contact with the finished water. While such bacteriological data suggest that colonization occurs on the surface of the encrustations, pictorial in situ evidence of this phenomenon has been unavailable. With the development and refinement of the scanning electron microscope (SEM), however, it is now possible to visually examine water main deposits for bacteria and other organisms.

Sample collection and preparation

Water utilities throughout the US were requested to provide water main encrustations when they became available during water main servicing, repair, or replacement. The cooperating utilities were provided with sterile plastic bags into which the samples could be placed after removal from the pipe interior, in order to maintain the moist condition within the encrustations. Addressed mailers expedited shipment to the laboratory. Following receipt of the samples, the specimens were refrigerated to stabilize and maintain the microorganisms present in the encrustation. The water utilities that provided encrustations for this study included the Cambridge (Mass.) Water Department, the Medford (Ore.) Water Commission, the New Haven (Conn.) Water Company, the Portland (Ore.) Bureau of Water Works, the Sioux Falls (S.D.) Department of Water, the Tacoma (Wash.) Water Division, and the City of Wilmington, Del. The system characteristics are presented in Table 1.

For SEM analysis the encrustations provided by the utilities required a series of dehydration steps to remove all water

TABLE 1
System characteristics of utilities providing water main encrustations

Utility	Source Water		Filtration	Disinfection (Chlorine Residual)		Microorganisms Found in Encrustations		
	Ground	Surface		Free	Combined	Algae-Diatoms	Bacteria	Actinomycetes
Cambridge, Mass.		•	•	•			•	•
Medford, Ore.	•			•		•	•	
New Haven, Conn.		•	•	•			•	•
Portland, Ore.		•			•		•	•
Sioux Falls, S.D.	•	•*	•	•		•		
Tacoma, Wash.	•	•		•		•		
Wilmington, Del.		•	•	•			•	

*Uses surface waters to meet peak demands only

Electron micrographs of encrustations revealed a hard but porous surface beneath which many kinds of crystal arrays were found.

Fig. 1. Surface sample from Portland magnified 3000 X

1.0 μm

Fig. 2. Interior sample from Medford magnified 1600 X

10.0 μm

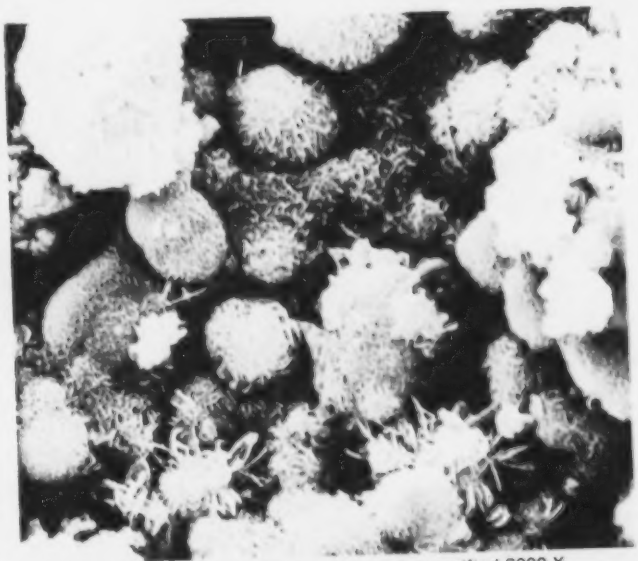

Fig. 3. Interior sample from Portland magnified 2000 X

10.0 μm

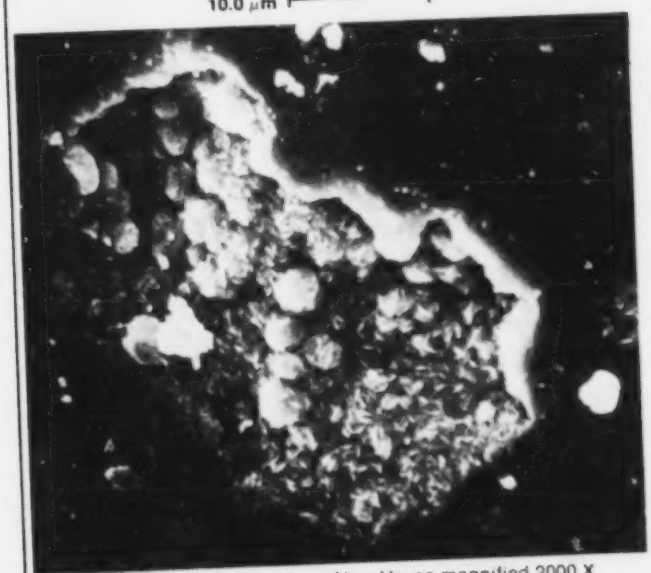

Fig. 4. Interior sample from New Haven magnified 2000 X

1.0 μm

Fig. 5 Bottom sample from Wilmington magnified 2000 X

1.0 μm

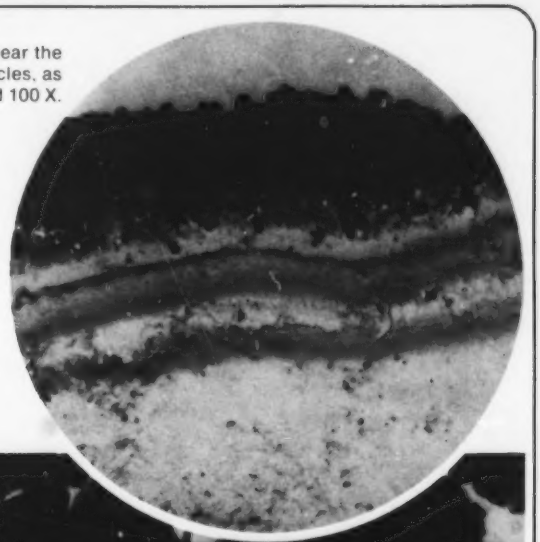

Fig. 6. Laminar deposits appeared near the encrustation surface of several tubercles, as in this surface edge sample magnified 100 X.

10.0 μm

Diatoms were present in several specimens, particularly those from utilities in the Northwest that do not use filtration in treating surface waters.

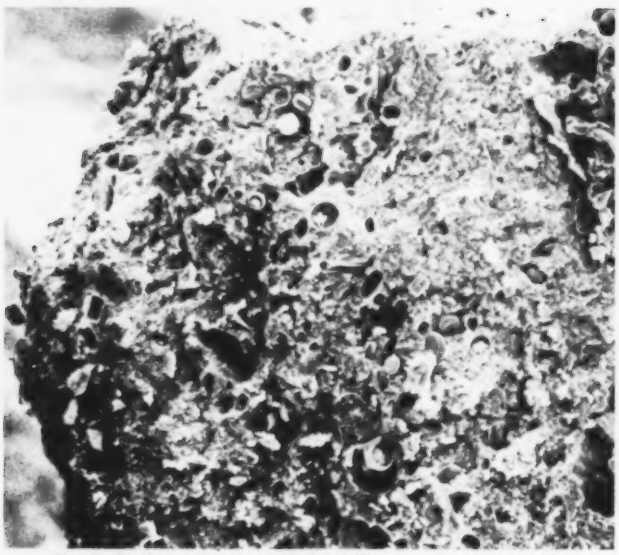

Fig. 7. Surface edge sample from Tacoma magnified 100 X

10.0 μm

Fig. 8. Surface sample from Tacoma magnified 2000 X

1.0 μm

Fig. 9. Surface sample from Medford magnified 500 X

10.0 μm

Fig. 10. Surface sample from Tacoma magnified 3000 X

1.0 μm

Algae were found in a sample from an unfiltered system (Fig. 11), while algae "microfossils" embedded within an amorphous material were identified in interior scans of other tubercle samples.

Fig. 11. Surface sample from Medford magnified 6000 X

1.0 μm

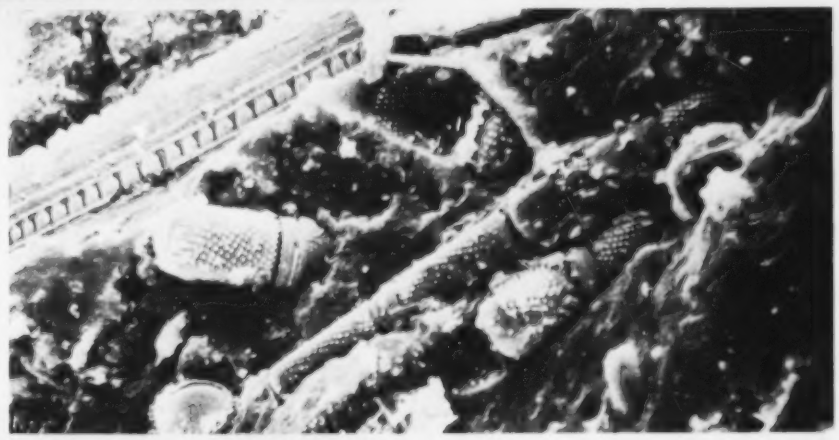

Fig. 12. Interior sample from Sioux Falls magnified 1400 X

10.0 μm

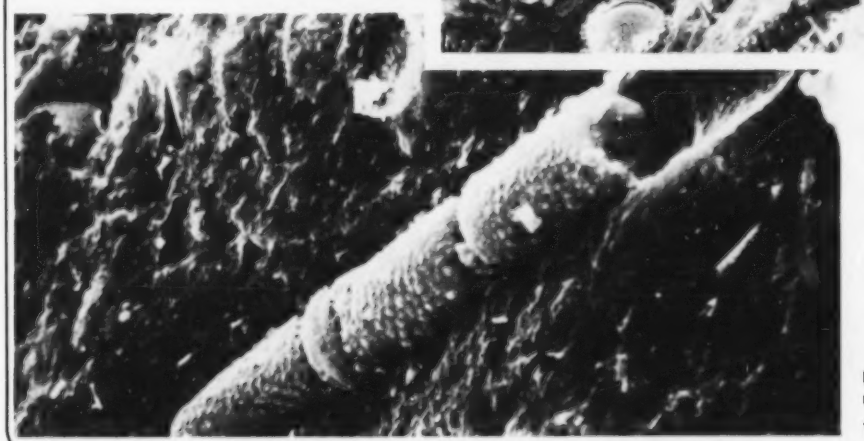

Fig. 13. Interior sample from Sioux Falls magnified 2000 X

1.0 μm

without destroying the physical integrity of the microorganisms. Dehydration first involved exposure of the specimen to ethanol concentrations of 50, 70, 95, and 100 percent. The ethanol was then sequentially replaced by immersing the specimen into increasingly higher concentrations of amyl acetate. Critical point drying using flowing anhydrous carbon dioxide in a pressurized chamber was employed to purge the dehydrated encrustation of the amyl acetate. Following this procedure the specimen was mounted with a silver collodion adhesive to an aluminum mounting stub. The dehydrated encrustations were then rendered electrically conductive by coating them with vaporized carbon and gold-palladium metal. The prepared encrustations were examined in an SEM microscope at a voltage of 20 kV.

Results

The encrustations examined were found to possess a number of common characteristics. Electron micrographs of the surfaces revealed a hard but porous surface (Fig. 1). Beneath the surface veneer, a multitude of crystal arrays was found (Fig. 2–5) with several encrustations exhibiting laminar deposits near the surface (Fig. 6). Microorganisms were predominantly seen at the surface or near the surface of most tubercles examined. Although it is not possible to determine from the electron micrographs which of the observed organisms were viable prior to solvent dehydration, the presence of large numbers of microorganisms exhibiting an identical physical morphology supports the argument that these organisms have colonized the surface of the tubercle. Such a conclusion appears quite reasonable since at the encrustation–water interface, nutrients and oxygen are constantly present and the embedded organisms are protected from residual disinfectant activity.

Diatoms or, more probably, their siliceous frustules were present in several specimens (Fig. 7–10), particularly those

Actinomycetes, noted for contributing to taste and odor problems, were tentatively identified on the surfaces of several encrustations.

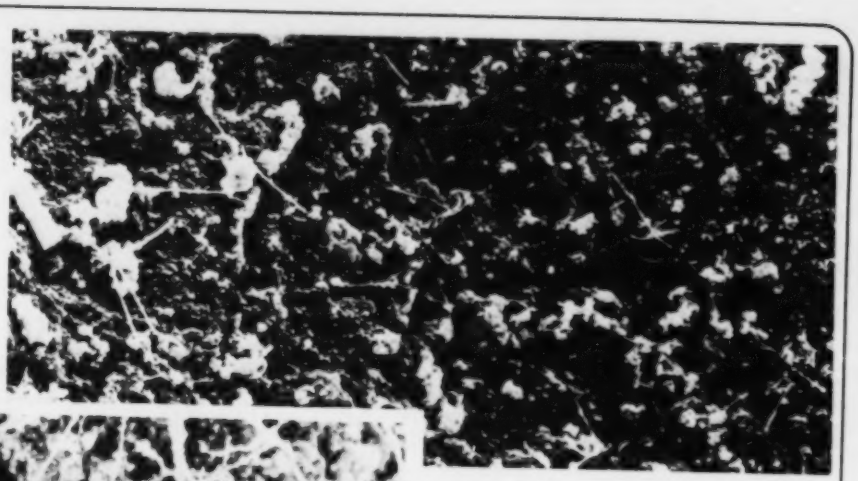

Fig. 14. Surface sample from Portland magnified 1000 X

1.0 μm

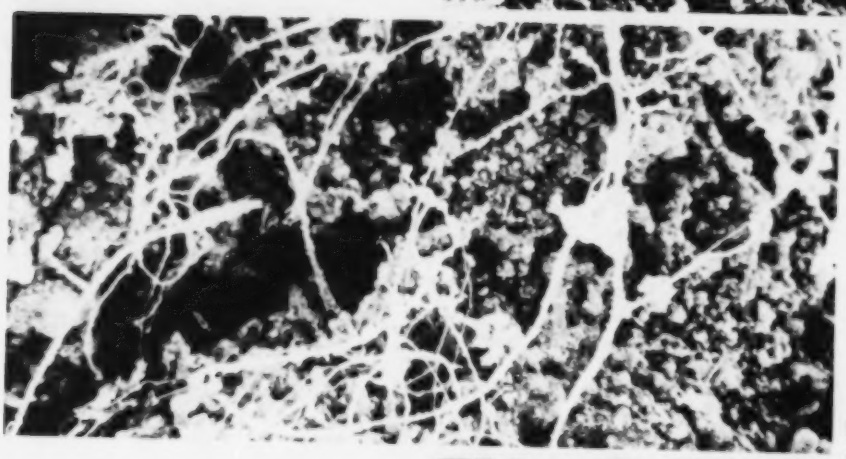

Fig. 15. Surface sample from Cambridge magnified 1000 X

10.0 μm

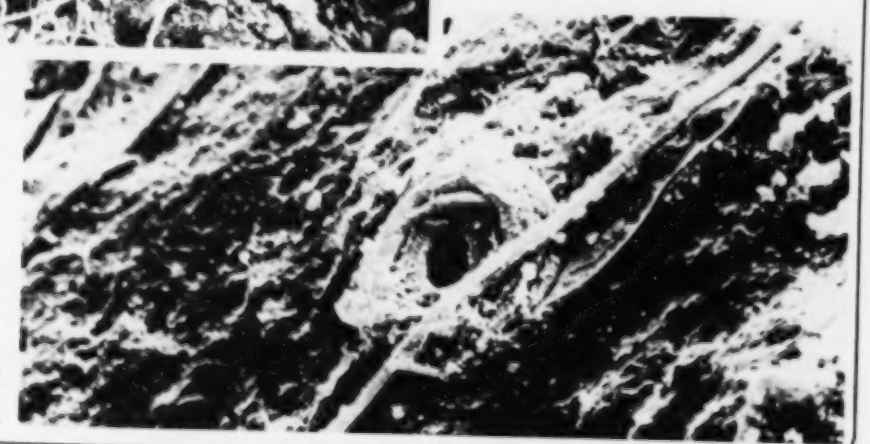

Fig. 16. Surface sample from Cambridge magnified 2000 X

1.0 μm

from the northwestern utilities in Tacoma and Medford. Such frustule accumulations may occur in systems that do not use filtration in the treatment of surface waters (Table 1).

Algae were also found in an unfiltered system (Fig. 11). Electron micrographs of the interior of a tubercle from Sioux Falls present evidence of the historical presence of algae in the distribution network, revealing the existence of "microfossils" of algae embedded within an amorphous material (Fig. 12, 13).

Actinomycetes, filamentous bacteria that are noted for contributing to taste and odor problems in drinking waters, have been tentatively identified on the surfaces of several encrustations (Fig. 14–16); taxonomic identification was based principally on morphology and size. Culturing of these same encrustations resulted in the development of white colonies that produced a water-soluble brown pigment around each colony. The production of such pigments is a specific characteristic for the actinomycetes.

Increasing sequential magnification of the surface of a porous specimen from New Haven demonstrated the ability of microorganisms to colonize within this environment (Fig. 17A–17D). Figures 17A and 17B show the surface texture of the intact tubercle, while Fig. 17C and 17D provide pictorial evidence of active bacterial colonization beneath the surface veneer; some bacteria appear to be embedded within a granular matrix (Fig. 17D). Obviously, enumeration by conventional bacteriological cultural procedures greatly underestimates the true bacterial populations present in these deposits. In another sequence of electron micrographs (Fig. 18A–18D), a variety of biological debris (diatoms) and curved, rod-shaped organisms are present at the surface.

Additional samples from Cambridge (Fig. 19A–19B), Portland (Fig. 20 and 21), Medford (Fig. 22 and 23), New Haven (Fig. 24A, 24B, and 25), and Wilmington

Increasing sequential magnification of the surface of a porous specimen from New Haven demonstrated the ability of microorganisms to colonize.

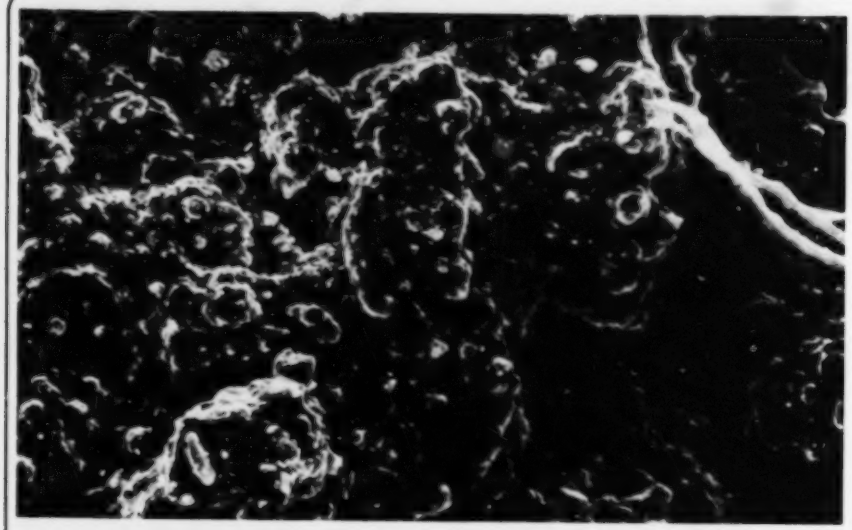

Fig. 17A. Surface texture of the intact tubercle magnified 400 X

10.0 μm

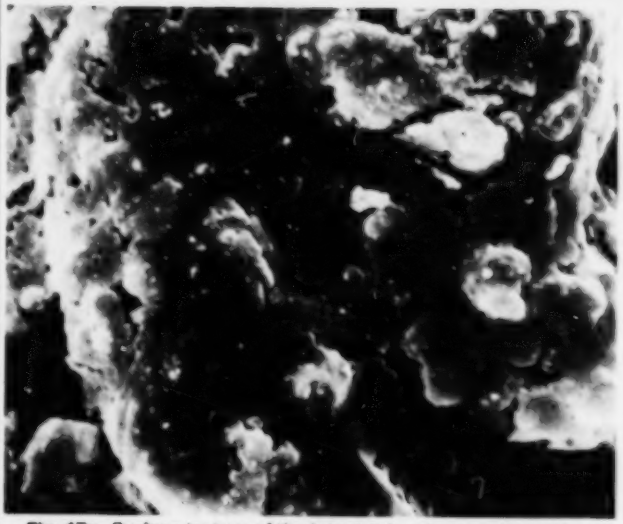

Fig. 17B. Surface texture of the intact tubercle magnified 2000 X

10.0 μm

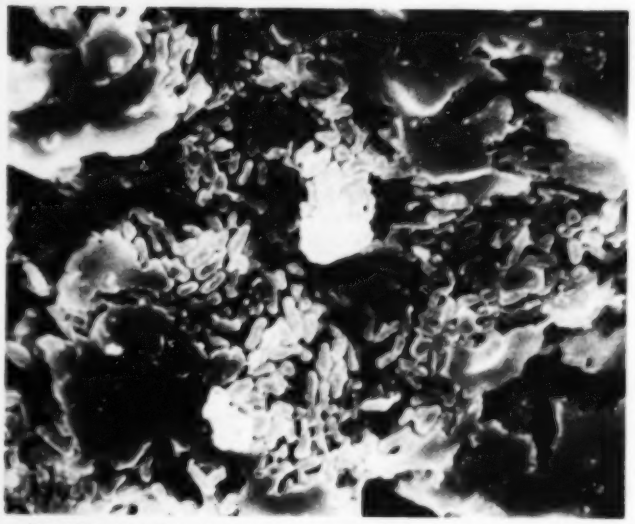

Fig. 17C. Active bacterial colonization beneath the encrustation surface veneer, magnified 3000 X

1.0 μm

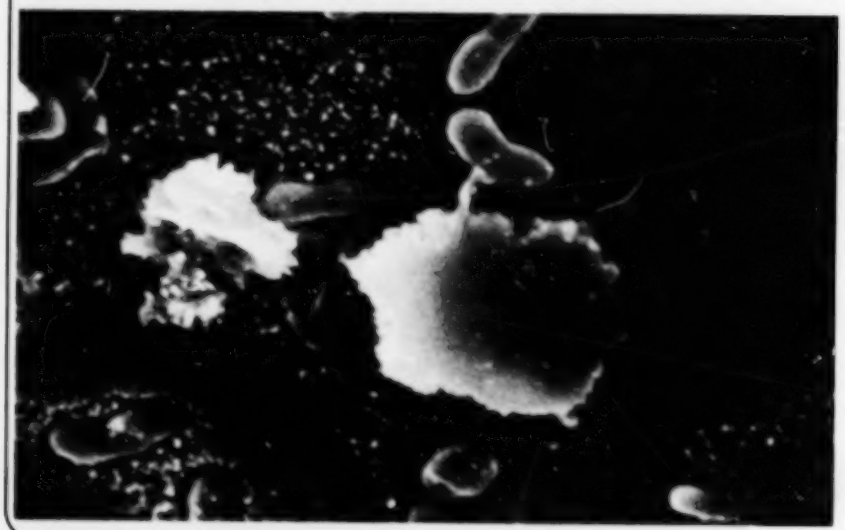

Fig. 17D. Bacteria embedded within a granular matrix, magnified 9000 X

1.0 μm

A variety of bacterial debris (diatoms) and curved, rod-shaped organisms were present at the encrustation surface of samples from Cambridge.

Fig. 18A. Surface sample from Cambridge magnified 1400 X

10.0 μm

Fig. 18B. Surface sample from Cambridge magnified 3000 X

1.0 μm

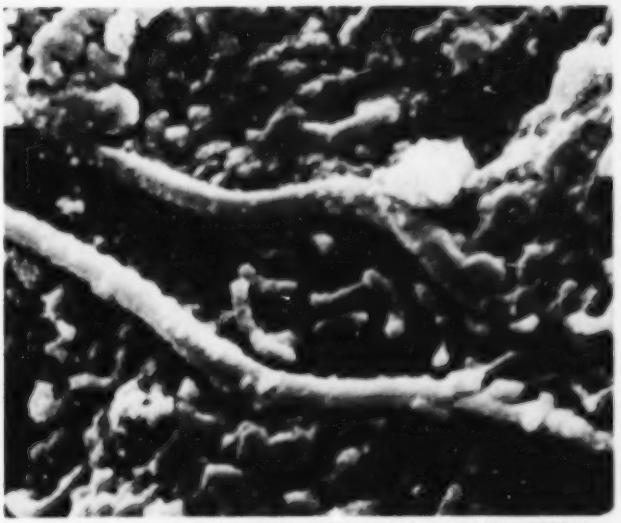

Fig. 18C. Surface sample from Cambridge magnified 7000 X

1.0 μm

Fig. 18D. Surface sample from Cambridge magnified 17 000 X

1.0 μm

A variety of other organisms appeared in additional samples taken from various utilities.

Fig. 19A. Elongated bacteria in encrustation surface sample from Cambridge magnified 4000 X

1.0 μm

Fig. 19B. Elongated bacteria in encrustation surface sample from Cambridge magnified 9000 X

1.0 μm

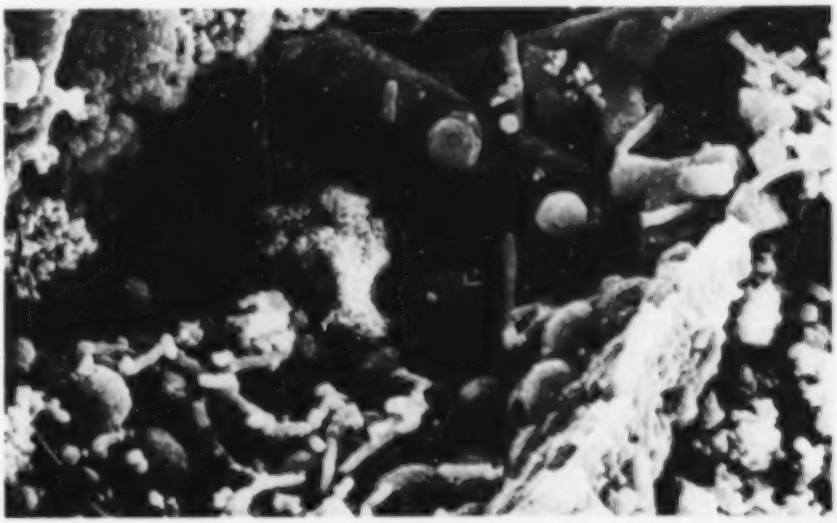

Fig. 20. Sample near encrustation surface from Portland magnified 3000 X

1.0 μm

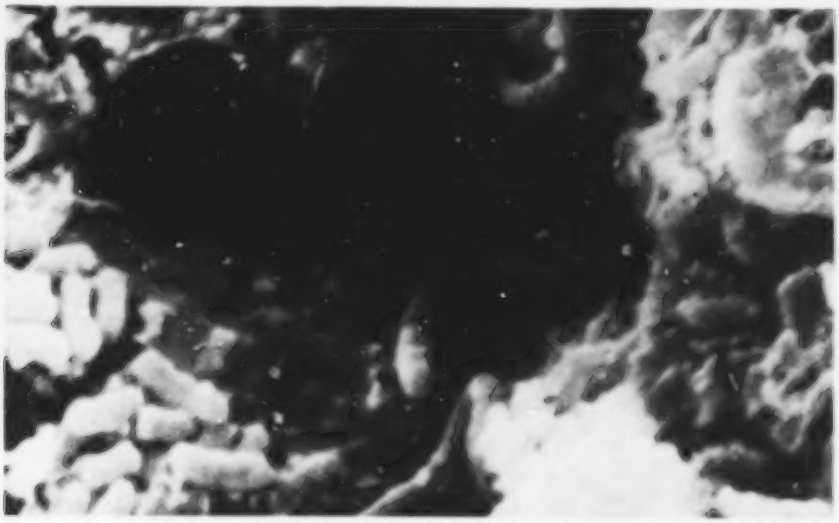

Fig. 21. Elongated bacteria in encrustation surface sample from Portland magnified 8000 X

1.0 μm

Fig. 22. Encrustation interior sample from Medford magnified 11 000 X

1.0 μm

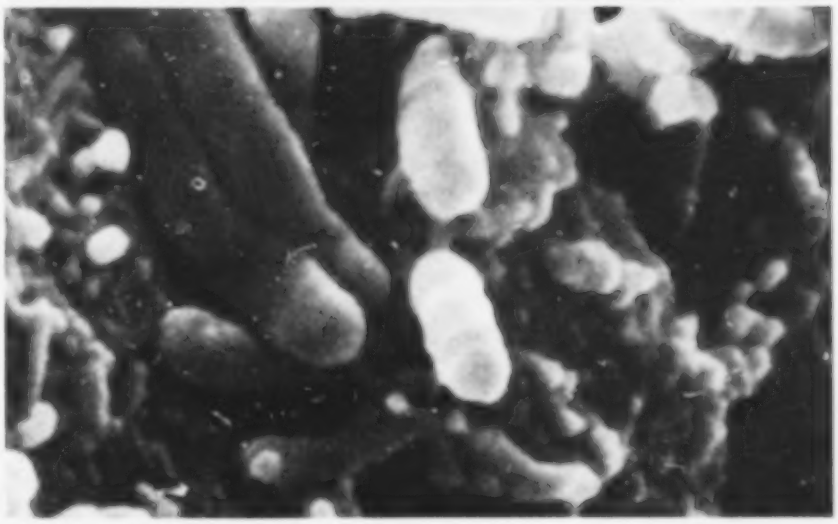

Fig. 23. Elongated bacteria in encrustation surface sample from Medford magnified 19 000 X

1.0 μm

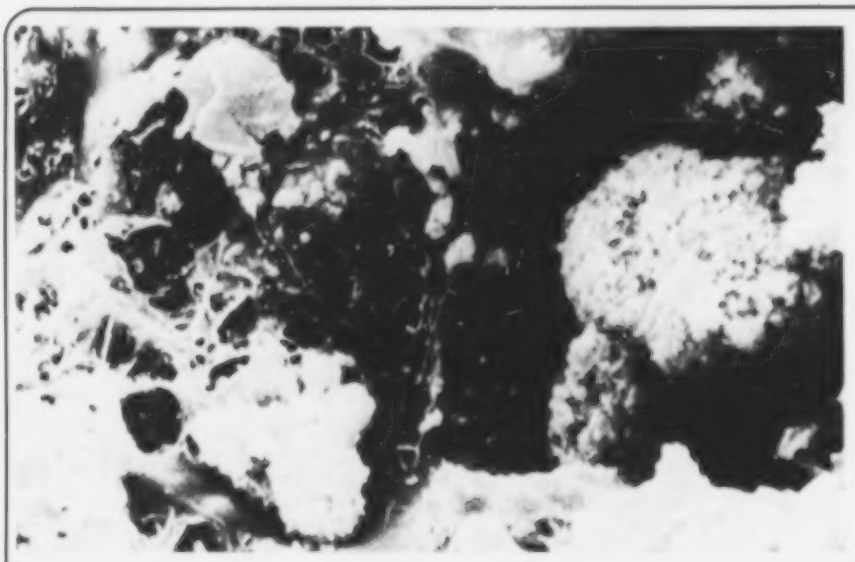

Fig. 24A. Filamentous bacteria in encrustation surface sample from New Haven magnified 3000 X

1.0 μm

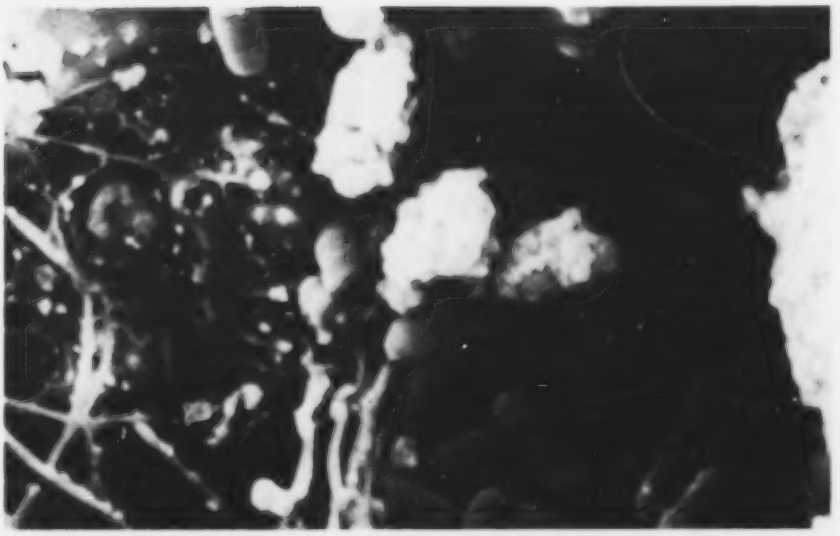

Fig. 24B. Filamentous bacteria in encrustation surface sample from New Haven magnified 11 000 X

1.0 μm

(Fig. 26) detail a variety of microorganisms, including elongated bacteria (Fig. 19A, 19B, 21, and 23), filamentous bacteria (Fig. 24A and 24B), and possibly flagellated cells (Fig. 25).

Discussion

Undoubtedly, the most significant information provided by this survey of water main encrustations is the magnitude of bacterial colonization occurring at the water interface. As can be seen from the electron micrographs, the scanning electron microscope provides an excellent method for in situ examination of microbiological activities in distribution main encrustations. The colonization of microorganisms and resultant high populations on or within the tubercle do not necessarily reflect inadequate treatment or disinfectant residuals but rather the inherent ability of microbial populations to adapt successfully to a variety of environmental variables. It was not possible to ascertain the growth rate of the microorganisms present in the encrustations or whether they were and are exposed to significant concentrations of disinfectant. Obviously, temperature, pH, oxygen concentrations, and available nutrient levels continually affect the viability of these organisms. It is also quite clear that earlier reported studies of bacterial populations in water main encrustations greatly underestimated the biomass within water distribution networks. The degree of bacterial quality changes can, however, be attenuated by producing water with lower particulate and nutrient levels and by minimizing sediment accumulations through effective corrosion control. Some of these problems can be managed through more effective water treatment practices, including the selection of adequate disinfectants at effective dosages. In severe cases, flushing, reconditioning, or replacing water mains and eliminating dead ends may be the only long-term solutions to continual contamination from bacterial populations protected by and multiplying within deposits that have developed in water mains over many years.

Acknowledgments

The authors wish to acknowledge the cooperation of Karen Siegfriedt, Robert L. Lee, Donald E. Jackson, G.E. Paulson, Les Hash, John A. Roller, and Hugo Victoreen in providing this laboratory with

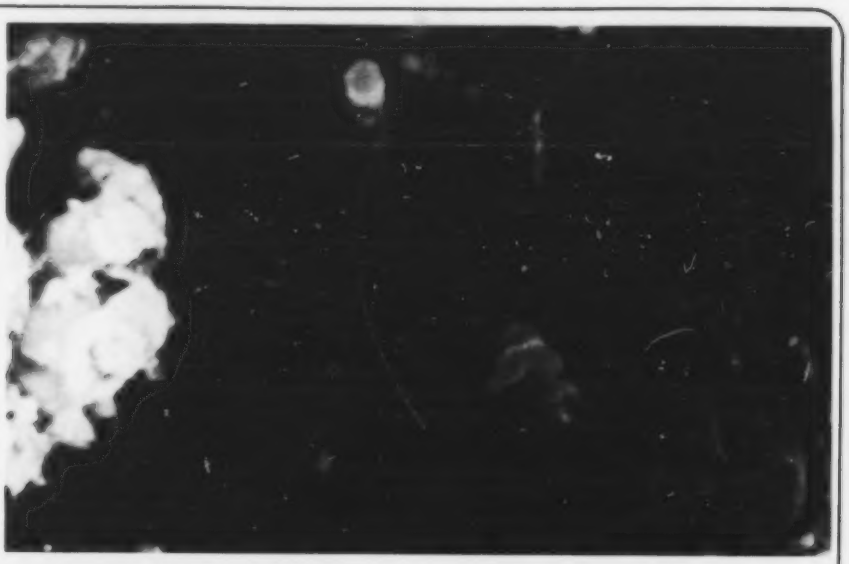

Fig. 25. Possibly flagellated cells in encrustation surface sample from New Haven magnified 12 000 X

1.0 μm

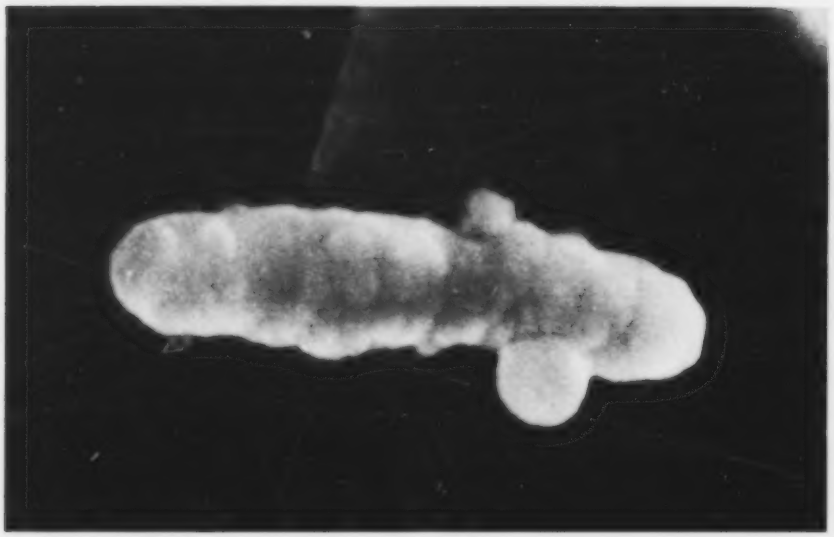

Fig. 26. Encrustation bottom sample from Wilmington magnified 22 000 X

1.0 μm

water main encrustations from the several water utilities. The technical support of James Millette and Helen Ball, USEPA, is also acknowledged.

References

1. GELDREICH, E.E. Is the Total Count Necessary? Proc. AWWA Water Quality Technol. Conf. (Dec. 1973).
2. ALLEN, M.J.; TAYLOR, R.H.; & GELDREICH, E.E. The Impact of Excessive Bacterial Populations on Coliform Methodology. Proc. AWWA Water Quality Technol. Conf. (Dec. 1976).
3. PETERSON, N. & FAVERO, M. Significance of Gram-Negative Bacteria in Water Supplies. Proc. AWWA Water Quality Technol. Conf. (Dec. 1975).
4. VICTOREEN, H.T. Soil Bacteria and Color Problem in Distribution Systems *Jour. AWWA*, 61:9:429 (Sep. 1969).
5. HUTCHINSON, M. & RIDGWAY, J.W. Microbiological Aspects of Drinking Water Supplies. *Aquatic Microbiology* (F.A. Skinner and J.M. Shewan, editors). Academic Press, New York (1979).
6. VICTOREEN, H.T. Water Quality Deterioration in Pipelines. Proc. AWWA Water Quality Technol. Conf. (Dec. 1977).
7. LEE, S.H. ET AL. Biologically Mediated Deterioration of Water Quality in Distribution Systems. Proc. AWWA Water Quality Technol. Conf. (Dec. 1977).
8. AINSWORTH, R.G. Discolored Water in Distribution Systems. Proc. AWWA Ann. Conf., Atlantic City, N.J. (1978).
9. RIDGWAY, R.G. ET AL. Water Quality Changes—Chemical and Microbiological Studies. Conf. Water Distribution Systems, Water Res. Centre, Medmenham, G.B. (1978).
10. O'CONNOR, J.T. ET AL. Deterioration of Water Quality in Distribution Systems. *Jour. AWWA*, 67:3:113 (Mar. 1975).
11. BAYS, L.R. Distribution. *Water Research Newsheet* 6 (Dec. 1975).
12. AINSWORTH, R.G. ET AL. Corrosion Products and Deposits in Iron Mains. Conf. Water Distribution Systems, Water Res. Centre, Medmenham, G.B. (1978).
13. ALLEN, M.J. & GELDREICH, E.E. Distribution Line Sediments and Bacterial Regrowth. Proc. AWWA Water Quality Technol. Conf. (Dec. 1977).

A water quality technology conference paper selected by the JOURNAL, authored by Martin J. Allen, envir. protection specialist, air and hazardous materials div., USEPA, Dallas, Tex., and Raymond H. Taylor (Active Member, AWWA), res. microbiol., and Edwin E. Geldreich (Active Member, AWWA), chief, microbiol. trtmt. branch, both of USEPA, Cincinnati, Ohio.

22299 4430

Printed in U.S.A.